

Evaluation of Pine Mortality in the Forests of Nicaragua

June 2008

By

Eckhardt, L.G., Menard, R.D., Ward, J.D., Sediles, A.



Evaluation of Pine Mortality in the Forests of Nicaragua

Abstract:

On a recent trip (April 4-21, 2008), Roger Menard and James Ward (USFS), and Dr. Lori G. Eckhardt (AU), traveled to Managua, Nicaragua at the request of Prof. Alberto Sediles, Entomologist for Universidad Nacional Agraria (UNA), for technical assistance in the evaluation of an ongoing disease/insect problem in their pine forest. The primary focus of the trip was a follow up to a previous trip made by Roger and Denny in September 2007 concerning an unknown cause of mortality in natural regeneration stands of *Pinus oocarpa* located in the Nueva Segovia district of Nicaragua. The first and second trip involved formal introductions to UNA rector and staff, Instituto Nacional Forestal (INAFOR) staff, and the Ministry of Natural Resources (MARENA) staff. Personnel from these departments accompanied Roger, Denny, and Dr. Eckhardt to the field to observe symptoms and collect samples for diagnosis. During the first trip, Roger and Denny visited two sites of interest; one with mortality in natural pine regeneration stands of *P. oocarpa* and another site with planted pines of *P. caribbea* heavily affected by a type of gall. The second trip with Dr. Eckhardt was a revisit to collect insect and root samples from the *P. oocarpa* stands exhibiting tree mortality. Professor Sediles was also interested in (1) assistance and training in field insect trapping techniques, (2) field and lab methods for collection and isolation of fungi from insects and tissue samples, and (3) urban forestry monitoring, diagnosis and management. Personnel from UNA accompanied Denny, Roger and Dr. Eckhardt on a trip around Managua to evaluate some of the urban forestry problems around the city. The trip concluded with a forest pathology seminar presented by Dr. Eckhardt and a tree pruning seminar presented by Roger to UNA staff and students. The diagnosis at this point is inconclusive for both symptomologies. Dr. Eckhardt's lab is currently conducting research into southern pine decline associated with *Leptographium* species in the southeastern United States. Dr. Eckhardt's lab did isolate a *Leptographium* from infected root samples but the fungal species is currently unidentified. These isolates have been sent to the laboratory of Dr. Michael Wingfield's, internationally recognized expert on *Leptographium* species, Mondi Professor of Forest Pathology, the University of Pretoria, South Africa, to be described and named. According to Dr. Wingfield, it is likely that this will be not only a new *Leptographium* species but there may also be a new insect vector identified when more insect trapping is conducted. Also, there is a continued effort to determine the cause of the extensive galling of *P. caribbea*. The cooperating agencies are still working on the final determination as to the casual pathogen and or insect involved with the pine mortality. Insect dispersal of these fungi also places a higher risk of introduction into other geographic regions or country. Invasive insects have in the past been associated with many introductions of other pathogenic organisms with devastating effects on the affected host. Causal determination, pathogenicity/virulence, rate of spread, and economic impact of this problem are greatly needed to assist the Nicaraguan agencies in reducing or preventing the escalating mortality of natural pine regeneration.

Background:

Nicaragua is the largest Central American country with borders on the Caribbean Sea and the Pacific Ocean. The nation has 65 different commercially valuable species of trees. Among the most valuable species are pine, rosewood, mahogany, and cedar. Nicaragua has 3.3 million hectares of forest cover (almost all classified as natural forest), mostly tropical hardwoods or mangroves. Only 453,977 ha are classified as pine forests, located primarily in the Caribbean region (north east, Nicaragua), and 63,193 ha located in Nueva Segovia along the border with Honduras. Both INAFOR and MARENA are involved in the protection of managed forests and protected areas of this department. This department of Nicaragua has suffered significantly from natural disasters over the past several years. From 1999 to January 2002, over 30,000 ha of Nicaragua's pine forest (*P. caribaea* and *P. oocarpa*) was lost due to *D. frontalis* and subsequent insect control measures. In April 2003, devastating wildfires erupted in the

treated region, spreading over 8,000 ha. The fires eliminated much of the natural pine regeneration that had developed since the beetle outbreak was controlled in 2001. A problem of pine mortality in the natural pine regeneration of these areas has occurred since these disasters. INAFOR forestry agents for the Nueva Segovia department reported to UNA that natural pine mortality is increasing and was becoming a concern for local landowners. This prompted Alberto Sedelis (UNA entomologist) to begin an investigation. He was unable to find an obvious insect association and contacted Denny Ward (Forest Service entomologist), who had previously aided with the prior SPB outbreak, for further assistance. Denny suggested a technical assistance visit by a Forest Service pathologist to determine if a pine disease could be the problem. Denny brought Roger Menard (Plant Pathologist, USFS, FHP) to determine if maybe a pathogen was involved. Roger collected insect and pine tissue samples and sent them to Dr. Lori Eckhardt's (Assistant Professor) laboratory at Auburn University's School of Forestry and Wildlife Sciences. A subsequent visit included Denny, Roger, and Dr. Eckhardt to collect additional samples. The preliminary laboratory results from both field visit collection periods determined that an unidentified *Ophiostoma*, *Grosmannia* teleomorph and a *Leptographium* anamorph from both the insect and pine tissue samples were possibly involved (description and identification are underway). No other pathogenic fungi were isolated from the samples collected. Ophiostomatoid species are found worldwide, are tree pathogens, and have a strong insect vector association. Their importance was first recognized in the 1970's and 1980's and more recently in the current decade as pathogenic fungi of conifer roots. There is still ongoing debate as to the virulence of these fungi, but more recent research is in favor of a virulent root pathogen. These fungi have sticky spores that are spread by insects and in some Ophiostomatoid species spread from infected root to healthy root by root to root contact (root grafting). The insect vector association poses a significant risk to the movement within the current pine geography. Insect dispersal of these fungi also places a higher risk of introduction into other geographic regions or country. Invasive insects have been associated with many introductions of other pathogenic organisms with devastating effects on the affected host. Causal determination, pathogenicity/virulence, rate of spread, and economic impact are greatly needed to assist the Nicaraguan agencies in reducing or preventing the escalating mortality of natural pine regeneration.

Invasive insects have in the past been associated with many introductions of other pathogenic organisms with devastating effects on the affected host. Invasive insects alone are reported to cause the loss of approximately 9% of forest products, amounting to a cost of \$7 billion per year (USBC 1998). In forests, two of the most serious plant pathogens are the chestnut blight fungus (*Cryphonectria parasitica*), and Dutch elm disease (*Ophiostoma ulmi*). Now chestnut trees have all but disappeared. Removal of elm trees devastated by *O. ulmi* costs about \$100 million/yr (Campbell and Schlarbaum 1994). Approximately \$2.1 billion in forest products are lost each year to non-indigenous plant pathogens in the United States. In the southeastern U.S., new reports of an invasive root-feeding insect and an association with Ophiostomatoid species that may also be invasive are being linked with extensive pine mortality within the region (Eckhardt unpublished). These current invasive issues are important examples of potential problems and are important incentives to further study the cause of pine mortality in Nicaragua. Identification of the pathogen and insect associated is crucial to begin the process of understanding the etiology which could be used to mitigate or prevent its potential introduction into the U.S. through trade. Trade with Nicaragua has been on the increase since 1992 with 68.6 million dollars in total import trade to a total of 560.1 million dollars total in trade as April 2008. The increase in trade poses a threat for potential invasion by plant, insect, or pathogen into the U.S. This can be accomplished through the mutually beneficial process of technical assistance from multiple US organizations (i.e., US Forest Service, Auburn University, USAID...etc) working together with multiple Nicaraguan organizations (i.e., UNA, INAFOR, MARENA) to apply an IPM system focused on the pine mortality problem but with goals that can cover other future pest issues and increase the technical capabilities of the pest management staff at UNA to assist in forest health issues in Nicaragua.

Objectives:

1. To assess the cause of pine mortality in northern Nicaragua. Identify possible predisposing factors, both pathogenic and physiogenic, contributing to the mortality.
2. To make specific recommendations on the operational pathology and entomology program needs for UNA, INAFOR, and MARENA. Provide suggestions on equipment needs and cost, and the approach needed to monitor, diagnose and manage forest disease problems in Nicaragua.
3. To develop and present to UNA, INAFOR, and MARENA a proposed process for assessing the health of the nation's forests.
4. To evaluate a number of other forest disease and urban forest problems and make recommendations for appropriate action.
5. To present the system on visual crown rating for forest health and certify two instructors for Nicaragua.

Investigating and Consulting Team:

Denny Ward, Entomologist, United States Forest Service, State and Private Forestry, Forest Health Protection, Atlanta, GA, 404-347-2989

Roger Menard, Plant Pathologist, United States Forest Service, State and Private Forestry, Forest Health Protection, Pineville, LA, 318-473-7287

Lori Eckhardt, Assistant Professor of Forest Pathology & Entomology, Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University, AL 334-844-2720

Alberto Sediles, Entomologist, Universidad Nacional Agraria, Managua, Nicaragua, 505-856-9872

Activities:

Field: Sampled several areas with affected stands in the Nueva Segovia district near Ocotal Nicaragua in September 2007 and April 2008. Root samples of symptomatic trees were collected and traps for insect vectors ran for one week during these time periods (Fig. 1a). Material from insect galleries was also collected to determine species of fungi present.

Laboratory: Root samples were plated in Nicaragua from September 2007 and April 2008. There were also root samples sent to Dr. Lori Eckhardt at AU under APHIS permit # P526P-08-00566. Root samples were plated at AU and unknown/unidentified Ophiostomatoid species were sent to Dr. Mike Wingfield, Forest and Agriculture Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa for sequencing. They hold the largest data base for Ophiostomatoid fungi in the world. Insects that were trapped in the field were identified and plated on water agar and isolates of Ophiostomatoid fungi subcultured at UNA.

Agreement for Taxonomy: We currently have a Memorandum of Agreement between Departamento de Proteccion Agricola y Forestal, Universidad Nacional Agraria, Managua, Nicaragua and the Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University for assistance in the identification of forest pathogens (limited to fungi) and insect pests.

Training: The staff involved in the Forest Pest Management and Forest Health Protection Program from the Department of Agriculture and Forest Protection at UNA was trained in proper root sampling in the field and processing in the laboratory (Fig. 1b). Staff was also trained in insect trapping and insect

processing for isolating fungal associates (Fig. 1a). Urban forestry tree assessment for insect and disease problems were also included (Fig. 1c).



Figure 1. a. Insect sampling; b. Insect and root work in the UNA laboratory; c. Urban forest diagnosis field trip.

Results & Findings:

Trip 1 (November 2007):

Field Observations: In the Nueva Segovia district near Ocotal Nicaragua, tree foliage was chlorotic and wilting (Fig. 2a). Roots and butt were stained and resinous and contained insect galleries and feeding areas. Black stain and resinous tissue extended from the roots up through the butt and into the stem. In Matagalpa Nicaragua, *P. caribaea* were infested with galls in branches and on the stem (Fig. 3). Galls ranged in size from golf ball to baseball size.

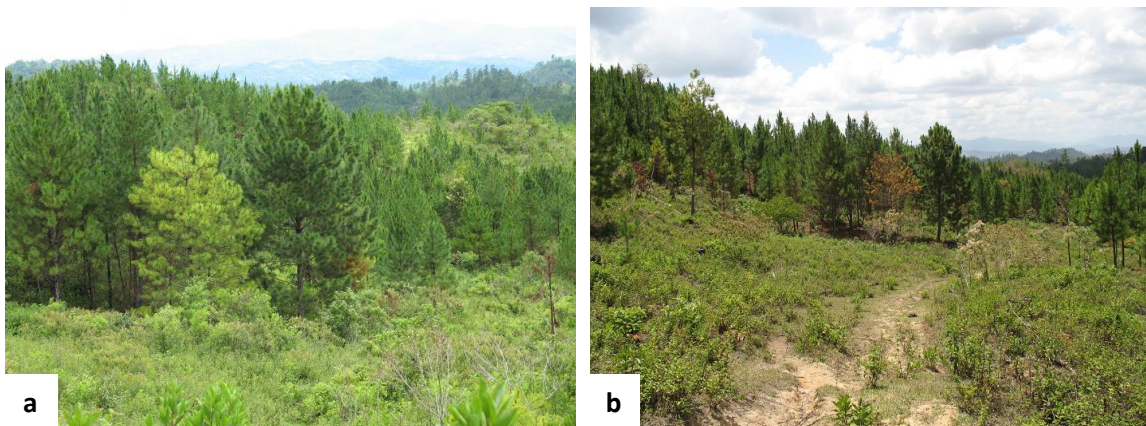


Figure 2. Field trip to Nueva Segovia district near Ocotal. Symptomatic *P. oocarpa* (a) yellow November 2007 and same symptomatic tree (b) dead April 2008.

Laboratory Observations: Fungi from the genera *Ophiostoma* / *Grosmannia* were isolated from root samples from symptomatic trees sent to AU. These isolates have been subcultured and put on slants as pure cultures. These are not species that have been previously isolated from the U.S. and are being sent to FABI for sequencing and comparison to the world library.



Figure 3. Galls in *P. caribaea* in Matagalpa Nicaragua.

Trip 2 (April 2008):

Field Observations: In the Nueva Segovia district near Ocotal Nicaragua, tree foliage was chlorotic and wilting, turning brown (Fig. 2b). Roots and butt were stained and resinous and contained insect galleries and feeding areas. Black stain and resinous tissue extended from the roots up through the butt and into the stem (Fig. 4). Saplings were found in clumps of 4 – 6 dead trees and roots, butts and stems were resinous and stained.



Figure 4. Staining and resinosis in stem and butt of *P. oocarpa*.

Laboratory Observations: *Ophiostoma* / *Grosmannia* and *Leptographium* were isolated from root samples from symptomatic trees sent to AU (Fig. 5). These isolates have been subcultured and put on slants as pure cultures. *Ophiostoma* / *Grosmannia* have also been isolated from *Dendroctonus approximatus* Dietz (Fig. 6) that were plated at UNA prior to being preserved in ethanol. These are not species that have been previously isolated from the U.S. and are being sent to FABI for sequencing and comparison to the world library. The *Ophiostoma* / *Grosmannia* isolated from the insects matches the morpho-type of the isolates from the roots.



Figure 5. Ophiostomatoid fungi isolated from *P. oocarpa* root tissue.



Figure 6. *Dendroctonus approximatus*

Recommendations:

Causal determination, pathogenicity/virulence, rate of spread, and economic impact of this problem are greatly needed to assist the Nicaraguan agencies in reducing or preventing the escalating mortality of natural pine regeneration. The proposed work below will answer many of these questions and get Nicaragua on their way to monitoring, diagnosing and managing their forest health problems. (*All recommendations are contingent upon further funding.*)

1. Study plots with insect traps need to be installed to determine what insects maybe involved and vectoring the isolated pathogenic fungi. These traps will need to be checked every two weeks for a period of 12 months. The insects will be plated on water agar prior to being preserved in ethanol. The plates and insects will be sent to Dr. Lori Eckhardt at AU for identification. Insect and insect plates received from UNA will be identified. Any unknown / unidentified Ophiostomatoid species will be sent to FABI.
2. Root samples need to be taken over a seasonal period. Dr. Eckhardt and Professor Sediles will take samples in July 2008. Professor Sediles will take samples in November 2008. Dr. Eckhardt, Roger and Denny will take samples in February 2009. Samples will be processed at UNA. Half of the samples will be sent to Dr. Lori Eckhardt at AU under APHIS permit # P526P-08-00566 to be plated and identified. Root samples taken in July 2008, November 2008 and February 2009 received from UNA be plated and identified. Any unknown/unidentified Ophiostomatoid species will be sent to FABI.
3. Competition assays will need to be carried out using the Nicaraguan isolates and the Ophiostomatoid fungi already reported in the U.S. to determine the native species ability to compete with an invader.
4. Pathogenicity experiments need to be completed on Nicaraguan and Southeastern U.S. species of pine to determine the impact that these root fungi may have in the southeastern U.S. ecosystem. Permits have been filled with APHIS to complete greenhouse seedling pathogenicity experiments using the Nicaraguan isolates and the southern pines (loblolly, shortleaf, longleaf, slash). If permits are unobtainable, southern pines will be sent to UNA and the experiments carried out there. The Nicaraguan isolates would be compared to the Ophiostomatoid fungi already reported in the U.S. (Native: *Leptographium procerum*, *L. terebrantis*; Invasive: *L. serpens*, *L. huntii*).
5. A tree fungal pathogen identification class is needed and tentatively set for January 2009 to train the Forest Pest Management and Forest Health Protection staff (and others, i.e., students) in the identification of fungal pathogens that affect forest trees (both urban and plantation).
6. A crown rating vigor class is needed and tentatively set for January 2009 to train UNA, INAFOR, and MARENA personnel.

Contacts & Affiliations:

Universidad Nacional Agraria (UNA)

Telemaco Talavera Siles, Rector

Martha Zamora, Department Head, Departamento de Proteccion Agricola y Forestal

Alberto Sediles, Entomologist (*Working Contact*)

Lucia Romero, Entomologist

Yanet Gutierrez, Plant Pathologist

Carolina Lopez, Plant Pathologist

Andres Lopez, Forest Engineer and GIS Specialist

Teresa Morales, Ecologist

Lucelia Gonzales Lopez, Student, Plant and Forest Protection Program

Fabiola Areas Sevilla, Student, Plant and Forest Protection Program

Intituto Nacional Forestal (INAFOR)

Zaida Zuniga, Head of Forest Pest Department

Nelson Hernandez, County Officer for Forestry

Fanor Cerna, County Agent

Ministerio del Ambiente y Recursos Naturales (MARENA)

Carlos Morales, County Officer for Forestry

Itinerary:

September 2007: Initial Evaluation: Denny and Roger

April 2008: Additional sampling: Denny, Roger & Dr. Eckhardt

July 2008: Culture checks and additional sampling: Dr. Eckhardt & Denny

August 2008: Establish 10 FHM plots (5 in mortality areas, 5 in healthy areas) for 12 month insect collection: Denny and Roger

January/February 2009: Train pathologist in fungal identification, additional sampling: Denny, Roger and Dr. Eckhardt

April/May 2009: Meet with Dr. Wingfield (FABI) and tour affected areas and discuss current results: Denny, Roger and Dr. Eckhardt

Acknowledgements:

We would like to thank the United States Forest Service, International Activities Team for funding travel needs for Denny Ward and Roger Menard, and Dean Richard Brinker and Associate Dean Graeme Lockaby of the School of Forestry and Wildlife Sciences, Auburn University for assistance in funding for travel and supplies for Dr. Lori Eckhardt. We would also like to thank George Matusick and Chris Pope for their many hours in the laboratory at Auburn University processing samples.

References:

Campbell FT, Schlarbaum SE. 1994. *Fading Forests: North American Trees and the Threat of Exotic Pests*. New York: Natural Resources Defense Council.

USBC. 1998. *Statistical Abstract of the United States 1996*. 200th ed. Washington, DC: U.S. Bureau of the Census, U.S. Government Printing Office.